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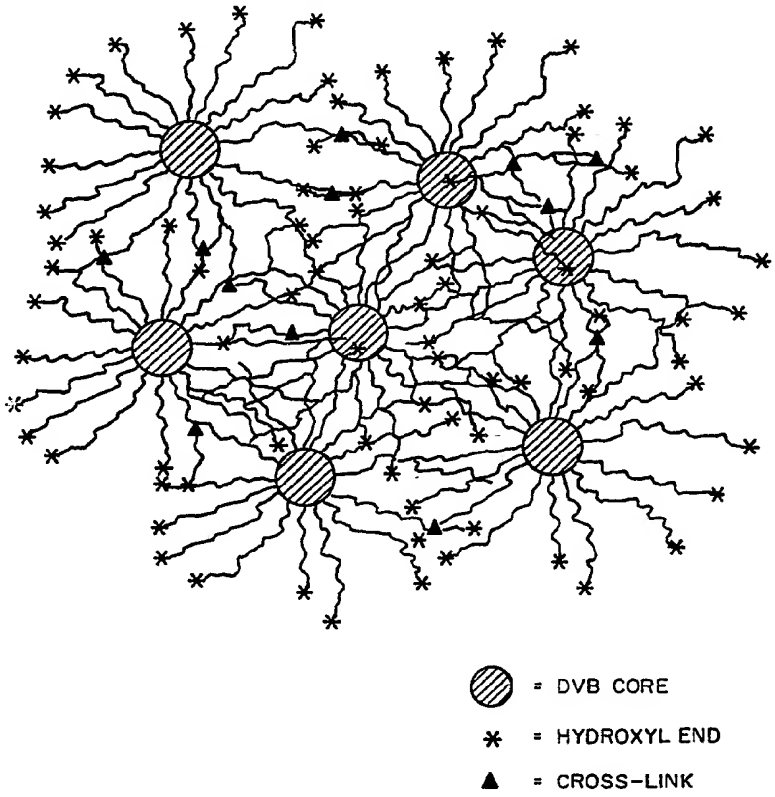
(54) Title: IMMOBILIZED POLYETHYLENE OXIDE STAR MOLECULES FOR BIOAPPLICATIONS

(57) Abstract

This invention pertains to a method for immobilizing polyethylene oxide (PEO) star molecules in the form of hydrogels. The PEO star molecules are biocompatible and demonstrate non-thrombogenic properties. As such, the PEO star molecules have numerous uses for biomedical applications. The hydrogels contain a high percentage of terminal hydroxyl groups for attachment of affinity ligands and can be used for separating and purifying therapeutic proteins.



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IMMOBILIZED POLYETHYLENE OXIDE STAR MOLECULES FOR BIOAPPLICATIONS

Background of the Invention

Polyethylene oxide (PEO) is an important biomaterial because it is non-adsorptive toward biopolymers, and is non-thrombogenic, i.e., it does not adsorb proteins of the intrinsic clotting system nor of the platelet membrane. However, when PEO is combined with other molecules at the surface, thrombogenicity may be enhanced. Okkema, A.Z., J. Biomat. Sci. 1:43-62 (1989). Thus, it is essential that no other molecular entity besides PEO be accessible to proteins. It has been widely studied as a blood-contacting biomaterial in various forms: in segmented polyurethanes, in block copolymers with styrene or siloxane blocks, end-linked into junctions through isocyanate reactions, as side-chains on acrylate polymers and as hydrogels cross-linked from PEO solutions.

PEO is naturally soluble in water and certain organic solvents. Therefore, in order to render PEO insoluble it must be crosslinked, or end-linked to a support. The manner in which this is accomplished often affects physical and chemical properties of PEO.

Chemical crosslinking of PEO can be employed but the chemical crosslinking agent (e.g., a polyglycidoxypropyl siloxane) may be incorporated into the PEO. This can cause adverse biopolymer reactions, including non-specific binding of proteins and platelet adhesion.

Physically crosslinked PEO produced from polyethylene oxide-polystyrene multiblock polymers or from polyether-urethanes suffers from the presence of the non-PEO material at the surface. Adverse biological reactions caused by the non-PEO material can be avoided if the molecular weight of the PEO is made higher than about 5000. However, such material tends to swell excessively in water and is fragile.

End-linking PEO to supports by various means, so as to leave an available hydroxyl group for attachment of an affinity ligand, for example, is not easily carried out if the molecular weight of the PEO is more than about 1000. Furthermore, complete coverage of a surface by end-linking PEO is very difficult, unless the molecular weight is relatively high (several thousand).

Various forms of PEO have also been widely used as a molecular leash for affinity ligands and enzymes. Golander, C.G. et al., Int. Chem. Congress of Pacific Basin Societies, Abstract No. 253, Honolulu, HI, December 17-22, 1989; Harris J.M., J. Macromolecular Sci. C25:325-373 (1985); Holmberg, K., Int. Chem. Congress of Pacific Basin Societies, Abstract No. 255, Honolulu, HI, December 17-22, 1989. Typically, PEO has terminal hydroxyl groups which can be activated for attachment to biopolymers. Most processes for forming PEO biomaterials, however, reduce the hydroxyl content to very low values or zero. In order to produce a crosslinked PEO having a significant concentration of terminal hydroxyls, low molecular weight PEO (2,000 to 10,000) are required but often result in fragile materials. Alternatively, using short PEO side chains

on macromonomers like polyethylene glycol methacrylate may result in exposure of the methacrylate residues at the surface.

Thus, a need exists for a method of immobilizing PEO to a support surface without detracting from its physical properties and biological compatability. In addition, it would be desirable to provide a material having a high concentration of hydroxyl groups for attachment to biopolymers.

Summary of the Invention

This invention pertains to a method for covalently immobilizing polyethylene oxide star molecules onto a support surface and to hydrogels produced by the method. The PEO star molecules are immobilized in the form of hydrogels using radiation or hydroxyl group activation. The resulting PEO hydrogels have a high concentration of terminal hydroxyl groups which are available for attachment to biospecific affinity ligands or to the support surface itself. As such, the immobilized PEO star molecules can be used as a tool for separating and purifying biological molecules, while greatly reducing or eliminating non-specific binding.

The PEO star molecule hydrogels also have non-thrombogenic properties which make them suitable for applications in which blood contact is required. They are highly biocompatible and have excellent mechanical durability for numerous biomedical applications, including intravenous catheters and implantable vascular prostheses. The hydrogels of this invention can be grafted onto a suitable contact lens material for the manufacture of contact lenses.

Brief Description of the Drawings

Figure 1a shows a Type I PEO star molecule having a divinyl benzene (DVB) core and PEO chains attached thereto.

Figure 1b shows a Type II PEO star molecule having a DVB core and PEO chains attached thereto by polystyrene (PS) chains.

Figure 2 shows overlapping PEO star molecules (Type I) which are crosslinked to each other by electron irradiation.

Figure 3 shows several PEO star molecules (Type I) covalently attached to a support surface by tresylated hydroxyl groups.

Figure 4 illustrates the attachment of a biopolymer (IgG) to the surface of immobilized PEO star molecules.

Detailed Description of the Invention

Polyethylene oxide star macromolecules have been previously described by Lutz, P. and P. Rempp, Makromol. Chemie 189:1051 (1988) and Gnanou, Y. et al., Makromol. Chemie 189:2893-2897 (1988), the teachings of which are incorporated by reference herein. The star molecules are synthesized by anionic polymerization from divinyl benzene (DVB), ethylene oxide and optionally styrene. They have a core of divinyl benzene (typically on the order of about 50 angstroms) from which a predetermined number of polyethylene oxide chains or "arms" are grown. The cores however can be of polymeric material other than divinyl benzene. The length of each PEO chain corresponds to its molecular

weight and typically range from about 1,000 to about 10,000. Preferably, each star molecule will have from about 6 to about 50 arms. Two variations of PEO star molecules are shown in Figures 1A and 1B and are described herein as Type I and Type II, respectively. Type I star molecules contain a plurality of hydroxyl-terminated PEO chains (hydrophilic) that are attached to a hydrophobic DVB core by non-hydrolysable carbon-carbon bonds. Type II PEO star molecules are of similar composition except that the PEO chains are attached to the DVB core via hydrophobic polystyrene (PS) chains.

The concentration of hydroxy-termini on the PEO arms can be determined in advance by selection of the gross concentration of star molecules and the number of arms carried by the molecule. For example, a star molecule of 100,000 molecular weight with 20 PEO arms has 20 hydroxyls. To obtain comparable hydroxyl concentrations with linear PEO polymers, the molecular weight would have to be lowered to 10,000. However, hydrogels made of cross-linked linear PEO of comparable molecular weight (MW 10,000) are very fragile.

The PEO star molecules can be immobilized or grafted onto a support surface of any geometry (e.g., particles, porous plastic cores, thin plastic film, biomedical device, contact lenses) using ionizing radiation. According to the method, PEO star molecules are dissolved or suspended in an aqueous solution (preferably water) in a concentration sufficient to provide enough star molecules to cover the support surface to desired thickness. Typically, a sufficient concentration will be around 5 to 15 wt/vol%. Type I

star molecules form optically clear homogeneous solutions in water, while Type II star molecules form faintly turbid to opaque suspensions, due to the presence of polystyrene. The resulting solution is then deposited onto the support surface, such as by spreading, rotating the support or centrifugation.

The star molecules are then crosslinked together by exposing them to electron beam radiation which results in the formation of a hydrogel network. The term "hydrogel" refers to a broad class of polymeric materials which are swollen extensively in water but which do not dissolve in water. Typically, the solution is exposed to electron radiation in the range of from about 1 to about 6 megarads, most preferably 4 megarads. Gamma radiation can be used as the radiation source but may result in the degradation of the star molecules. Crosslinking occurs randomly between segments of the PEO arms, thus allowing the terminal hydroxyl groups to remain available for subsequent activation, such as coupling affinity ligands to the PEO arms.

Figure 2 shows several Type I PEO star molecules crosslinked together by electron radiation. The resulting hydrogel layers are of variable thickness but are typically on the order of magnitude of >1μM. The thickness of the hydrogel layer can be regulated by various techniques, such as doctor-blade spreading on a support web or centrifugal casting in tubes.

An advantage of electron radiation crosslinking is that the crosslinking reaction proceeds very rapidly, at a rate of approximately 1 foot/sec. in the case of web coating. The reaction proceeds by free-radical

coupling to produce a pure product. As such, the crosslinking reaction does not alter the chemical composition of the star molecules. Other known crosslinking techniques tend to introduce chemical components which may subsequently affect its biocompatibility. Further, the hydrogel network has a surface for contacting biological materials (eg. blood) which is essentially PEO chains. As such, the DVB and PS components are inaccessible or not recognizable to these biological molecules.

The resulting hydrogels have significantly greater mechanical strength than hydrogels formed from ordinary linear PEO having the same range of molecular weight as the star (i.e., 100,000 to 300,000). A gel made from 10 wt.% of 100,000 molecular weight linear PEO under identical dosage would have 2 to 10 times lower tensile strength than the network formed from star molecules, and would have only 1/10th the number of hydroxyl groups per unit area of surface. The concentrations of hydroxyl ends obtained by stars would translate to linear polyethylene oxide of around 5,000 mol. wt. or less. Such low molecular weight polymers cannot be crosslinked at all, or form gels of low strength with considerable soluble fraction.

In another embodiment, the star molecules can be covalently immobilized to a support surface by tresylation of the terminal hydroxyl groups. The support surface and star molecules are each pretreated prior to immobilization. As such, the support surface should contain active functional groups for immobilizing tresylated star molecules thereto, such as amino and/or

thiol groups. Likewise, the star molecules should be tresylated in an appropriate solvent at pH 10 or above, prior contacting with the support surface. Tresylation is particularly convenient since the PEO is solvated by media appropriate to tresyl chloride (e.g., dichloromethane, chloroform). This method results in a monolayer coating of the hydrogel over the support surface.

According to this method, an organic solution comprising PEO star molecules is exposed to tresyl chloride, under conditions such as to fix the tresyl groups to hydroxyl-termini on the star molecules. The resulting tresylated PEO star molecules are then transferred from the organic solvent to an aqueous solution. The pH of the aqueous solution is then adjusted to about 10 or above, so as to favor reaction with amino and/or thiol groups on the support surface. The pH-adjusted solution is contacted with a pretreated surface support that contains amino and/or thiol groups, under conditions whereby the star molecules become covalently bound in a dense layer to the support surface.

This process is further described below by way of illustration. For example, a Cellophane™ (cellulose containing plasticizers) containing support is placed in a bath of tetrahydrofuran and tresyl chloride. The hydroxyl groups on the surface of the Cellophane™ are then tresylated. Once tresylated, the Cellophane™ is aminated in a water solution of mercaptoethanol amine (pH 10) which results in binding the group $-SCH_2CH_2NH_2$ to the activated hydroxyl groups. Likewise, star

molecules are tresylated and then placed into an aqueous buffer (pH 10) containing the aminated Cellophane™. After a period of time (approx. 1 hr), the Cellophane™ is removed from the solution and rinsed to wash off any unbound star molecules. The star molecules become bound to the amino group via the tresylated hydroxyls. Figure 3 shows several PEO star molecules immobilized on a support surface. The attachment results from the reaction of amino groups on the support surface with tresylated hydroxyls on the star molecules.

The star molecule hydrogels can be covalently bonded onto an appropriate support surface using the methods previously described to thereby protect the support from recognition by biopolymers. A monolayer coating of PEO star molecules can be accomplished by attaching one or more PEO arms to the support. The remaining arms remain available for attaching biopolymers or affinity ligands. The PEO-coated support surface can then be exposed to a biopolymer having amino or thiol groups which can couple to available tresylated hydroxyl groups. These available groups function as molecular leashes or tethers for the biopolymer. For example, anti-Protein C antibody can be attached to the star molecules and will be selective for its antigen, Protein C. The PEO monolayer prevents adsorption of the biopolymers onto the support surface and can thereby reduce or eliminate non-specific binding of undesired biopolymers. Figure 4 demonstrates the use of star molecules for attaching affinity ligands, such as Immunoglobulin G. The symbol ◆

represents a covalent linkage between a PEO arm and an amino group on the support; ● represents a covalent linkage between a PEO arm and an amino or thiol group on IgG; ★ represents an endcapped previously tresylated hydroxyl (e.g., by treatment with mercaptoethanol).

Due to the number of available PEO arms which can accommodate ligands, the hydrogels of this invention can be used to continuously separate, purify and concentrate therapeutic proteins. Processing of the proteins will require cycles of coupling and decoupling of the ligate to affinity ligands bound to the stars.

The affinity surface can be of any geometric shape, such as particles packed in beds, freely moving particles and porous membranes. The hydrogels can be coated onto silica particles. In this case, polyethylene oxide is physically adsorbed to the silica surface but cannot be covalently bound unless the silica has been previously modified. Nonetheless, the polyethylene oxide hydrogel forms a shell covering the particle and it thus cannot escape. The hydrogels can also be deposited into pores of ultrahigh molecular weight, high density polyethylene such as Porex™ (Auburn, Georgia), on the surface of Goretex™ e-PTFE (expanded polytetrafluoroethylene) and Mylar™ film.

In most cases, once a PEO hydrogel is coated onto the affinity surface, the terminal hydroxyl groups are activated by tresylation. Preferably, this is accomplished by contacting the hydrogel with tresyl chloride dissolved in an organic solvent, such as dichloromethane. The tresylated PEO star molecules are then placed in buffered aqueous solution containing the

affinity ligand which is to be bound. Examples of preferred ligands include antibodies and F_{ab} fragments thereof, Protein A, active polysaccharides, heparin-NH_2, anti-Protein C IgG, and the F_{ab} fragment of anti-Protein C IgG.

Following affinity bonding of a specific ligate to its bound ligand, the hydrogel-coated affinity support is washed to remove unbound proteins. Remaining bound proteins are then decoupled by changing the composition of the eluting buffer, for example by changing the ionic strength or the pH (e.g., to pH 10 or above) of the eluting buffer. For example, a 1 M NaCl decoupling solution can be used in the case of antithrombin III bound to heparin. The decoupling results in free ligate in the eluting buffer. The ligate can then be separated from the eluting buffer using known techniques, such as by diafiltration described by Herak and Merrill, Biotech. Prog. 5:9-17 (1989). Separated ligates can then be concentrated using known techniques. Examples of some specific ligates include macromolecules, monoclonal antibodies, antigens, viruses and cells (e.g., blood platelets, white blood cells, endothelial cells and other non-blood cells).

In addition to bioseparations, the hydrogels made according to this invention are useful for a variety of biomedical applications, due to their non-thrombogenic properties and excellent mechanical durability. They are suitable for in vivo applications in which blood contact is required, including blood contacting implantable vascular prostheses, angioplastic stents, cardiovascular sutures, metabolic support catheters,

angioplastic balloon catheters, intraaortic balloon pumps, pulmonary artery catheters, artificial hearts and ventricular assist devices. The hydrogels may also be used for ex vivo devices, such as hemodialysis membranes and membranes for extra-corporeal oxygenators.

A preferred application for the star molecules of this invention is in the manufacture of contact lenses. PEO star molecules can be grafted onto a suitable art recognized contact lens material, such as gas permeable PEO, using the techniques described herein. For example, the contact lens material can be immersed in a PEO star molecule solution and exposed to ionizing radiation to thereby graft the star molecules onto the contact lens surface. Alternatively, the surface of the contact lens material can be modified by creating amino or thiol groups on its surface. The modified lens material is then exposed to activated PEO star molecules, such as tresylated star molecules described above. Due to the properties of the star molecules, absorption of proteineous deposits from natural enzymatic secretions of the eye by the star molecule coated-contact lens material can be eliminated or substantially reduced. Thus, the coated lenses will not become clouded or opaque because of lowered protein absorption.

Additional chemical components can be incorporated into the star hydrogels depending upon the application. In some instances it may be advantageous to incorporate heparin into the hydrogel to further reduce thromogenicity. While heparin can be attached covalently to tresylated hydroxyls on the star molecules, it is also

readily incorporated at high concentrations in the hydrogel by simply adding it to the solution of the star before irradiation. In this form it elutes into the blood flow over a significant period of time.

The invention will be further illustrated by the following Example.

Exemplification

Synthesis and Characterization of Various PEO Hydrogels

Linear PEO and various forms of star molecules having the physical properties described below were electron beam irradiated, at a dose rate of about 0.1 megarad per second, and with a 2 megarad dose per pass under the beam to form hydrogels. Radiation was delivered from a 3 MeV Van de Graaff generator (MIT High Voltage Research Laboratory).

Table 1 presents the apparent swelling ratio q at 25°C (q = volume of hydrogel equilibrated in water/ volume of original mixture irradiated) as a function of radiation dose D in megarad, and as a function of the star type. Two linear PEO samples are included for reference. The concentration of the solution as irradiated in every case was 10.0 wt/vol.% in MilliQ^R water. From Table 1 it is apparent that the swelling ratio q of hydrogels formed from star molecules is significantly less than for hydrogels from linear PEO types. Furthermore, the high styrene content Type II hydrogels (3103, 3229) exhibit virtually no swelling.

TABLE 1

Swelling Ratios q of 10 wt/vol.% Polymer/Water After Electron Beam Irradiation

Linear PEO	D	q	[OH] μM
Nominal 300,000 m.w.	4	2.03	0.33
	6	1.92	0.35
Nominal 100,000 m.w.	4	2.8	0.71
	6	2.4	0.83

Type I Stars (no styrene) mol. wt. total	# arms	M_{PEO}	D	q	[OH] μM
3098 229,000	43	5300	4	1.3	14.6
3210 142.000	40	3460	4	1.4	20.0
3224 79,000	8	10,000	6	1.6	6.3

Type II Stars mol. wt. total	%S	#arms	M_{PEO}	M_{PS}	D	q	[OH]
3103 190,000	20	16	8000	2000	4	~1.0	8.4 μM
3229 257,000	30	25	6800	3200	4	~1.0	9.6
3385 371,000	2	30	12,000	520	4	1.7	4.7
					6	1.6	5.7

D: dose in megarads
Total mol. wt. of stars by light scattering
q: Swelling Ratio
[OH]: g. equiv. per liter of gel swollen to equilibrium in water at 25°C.

From the results, the random cross-linking of star molecules cannot be expected to lead to networks like those produced from randomly cross-linked linear macromolecules, in which the functionality of the junction ϕ is necessarily 4. In contrast, the incorporation of stars implies incorporation of junctions of

high functionality ϕ, i.e., ϕ = # arms. Further, the "junction" is in effect a high modulus poly DVB core, in Type I stars, and an even more complicated entity, i.e., poly DVB with short polystyrene arms, in Type II stars. Thus, the space occupied by the "junction", and the thermodynamically adverse junction-water interaction place the star hydrogel beyond the tenets of the Flory-Huggins theory of swelling of randomly crosslinked networks.

The last column in Table 1 shows the molar hydroxyl content of the gel at equilibrium in water [OH], calculated as: (mols OH/100 g. dry polymer)q^{-1}, wherein the first term is determined as (number of arms/total mol. wt.)•100. Each original solution at 10 wt/vol.% contains 100 g dry polymer per liter. The final wt/vol.% polymer in the get at equilibrium with water is thus 10/q. This is very important if the star hydrogel is to be deployed as a model biomaterial to which bioactive species are to be grafted. It is desirable to have a high value of [OH] and a low swelling ratio q in order that the biomaterial remain approximately in the shape in which it was cast. Stars 3098 and 3210 as hydrogels provide examples.

In the hydrated state, i.e., in equilibrium with blood plasma, preliminary studies of platelet deposition indicate that the surface of star hydrogel is entirely PEO, that is, the poly DVB core is buried and inaccessible, because of the fact that the Star hydrogel acts as if it were a hydrogel of linear PEO. Crosslinking of these arms is random. Granting that all PEO arms have approximately the same molecular weight on a given star type as a consequence of the

anionic polymerization route. Under an electron beam hydroxyl radicals created from water constitute the principal reagent and therefore the PEO rather than the poly DVB and PS experiences macroradical formation and subsequent coupling. To some degree scission of the arms must occur competitively with cross-linking under radiation. The terminal hydroxyl concentrations [OH] calculated in Table 1 do not take this into account.

Biocompatability

Hydrogels containing Type I Stars 3098 or Type II Stars 3385, described above, were examined for biocompatability.

Tubular specimens of hydrogel were prepared from 10 wt/vol.% solutions of star polymers 3098 and 3385 using 0.7 ml of solution centrifugally cast and irradiated under 6 megarads inside glass tubes of 10 cm length x 9 mm lumen. These were tested in an ex vivo shunt model [indium 111 labeled platelets, baboon] with uncoated glass tubes as control. Over a period of 1 hour at a blood flow rate of 100 ml/min., there was no increase of indium count above background for the two hydrogel surfaces, whereas in glass control tubes (no coating) the count more than trebled over background.

Using similar techniques, glass tubes lined with 0.7 ml hydrogels formed from 10 wt./vol. % solutions of linear PEO of 100,000 and 300,000 mol. wt., respectively, under the same dose were prepared. Upon equilibration at 25°C with pure water, the apparent swelling ratios (final volume:initial volume) were : 1.3, 1.3, 2.8 and 2.0 for Star 3098, Star 3385, PEO 100,000 and PEO 300,000 hydrogels, respectively.

Values of 1.3 as compared to 2 or more mean that the star polymer based hydrogels when exposed to blood do not expand to such a degree as to compromise attachment to the surface on which they were cast. The lack of platelet uptake indicates that the star polymers in hydrogel form present a "pure" PEO surface to blood. As such, the DVB cores were shielded from access of plasma proteins by the PEO arms.

Equivalents

Those skilled in the art will recognize, or be able to ascertain, using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following Claims.

CLAIMS

1. A method of immobilizing polyethylene oxide star molecules to a support surface in the form of a hydrogel, comprising the steps of:
   a) providing a solution comprising polyethylene oxide star molecules having a plurality of hydroxy-terminated polyethylene oxide chains attached to a polymeric core;
   b) depositing the solution onto a support surface; and
   c) exposing the star molecules to conditions sufficient to produce a hydrogel which is immobilized to the support surface.

2. The method of Claim 1, wherein the star molecules comprise from about 6 to about 50 hydroxy-terminated polyethylene oxide chains attached to a polymeric core that is divinyl benzene.

3. The method of Claim 1, wherein step (c) is performed by irradiating the solution to produce a hydrogel of crosslinked star molecules; wherein the solution is an aqueous solution.

4. The method of Claim 3, wherein the aqueous solution is irradiated by electron beam radiation.

5. The method of Claim 1, further comprising the step of tresylating the star molecules in an aqueous solution at a pH of above about 10 prior to step (b); and wherein the support surface contains active functional groups for immobilizing the tresylated star molecules thereto.

6. The method of Claim 5, wherein the functional groups are thiol, amino or both.

7. A method of immobilizing polyethylene oxide star molecules to a support surface in the form of a hydrogel, comprising the steps of:

a) providing an aqueous solution comprising polyethylene oxide star molecules having a plurality of hydroxy-terminated polyethylene oxide chains attached to a divinyl benzene core;

b) depositing the aqueous solution onto a support surface; and

c) irradiating the solution with electron radiation under conditions sufficient to produce a hydrogel of crosslinked star molecules which is thereby immobilized to the support surface.

8. The method of Claim 7, wherein the support surface is selected from the group consisting of in vivo blood contacting vascular prostheses, angioplastic stents, cardiovascular suture, metabolic support catheters, angioplastic balloon catheters, artificial hearts and ventricular assist devices.

9. The method of Claim 7, wherein the support surface is selected from the group consisting of hemodialysis membranes and membranes for extracorporeal oxygenators.

10. A product produced by the method of Claim 7.

11. A method of immobilizing polyethylene oxide star molecules to a support surface in the form of a hydrogel, comprising the steps of:

a) exposing an organic solution comprising polyethylene oxide star molecules having a plurality of hydroxyl-terminated polyethylene oxide chains attached to a divinyl benzene core to tresyl chloride under conditions such as to fix tresyl groups to the hydroxyl termini;

b) transferring the tresylated polyethylene oxide star molecules from the organic solvent to an aqueous solution;

c) adjusting the pH of the aqueous solution to about 10 or above; and

d) contacting the solution of step (c) with a support surface containing amino and/or thiol groups for immobilizing the tresylated star molecules thereto, under conditions whereby the star molecules are covalently bound in a dense layer to the support surface.

12. The method of Claim 11, wherein the support surface is selected from the group consisting of particles, porous polymeric membranes, polymeric film, ultrahigh molecular weight high density polyethylene and biomedical devices.

13. The method of Claim 11, wherein the support surface is selected from the group consisting of in vivo blood contacting vascular prostheses, angioplastic stents, cardiovascular suture, metabolic support catheters, angioplastic balloon catheters, artificial hearts, ventricular assist devices, hemodialysis membranes and membranes for extracorporeal oxygenators.

14. A product produced by the method of Claim 9.

15. The method of Claim 11, further comprising the steps:

e) washing the support surface to remove any non-bound star molecules, leaving the tresylated polyethylene oxide star molecules remaining bound thereto;

f) contacting the support surface after step (e) with an affinity ligand of interest having amino and/or thiol groups thereon, under conditions such as to bind the ligand to the polyethylene oxide chains.

16. The method of Claim 15, wherein the affinity ligand is selected from the group consisting of antibodies, Protein A, F_{ab} fragments of antibodies and active polysaccharides.

17. The method of Claim 16, wherein the active polysaccharide is heparin.

18. A method of separating and purifying a ligate of interest, comprising the steps of:

a) providing a support surface having coated thereon, a hydrogel comprising polyethylene oxide star molecules having a plurality of ligand-terminated polyethylene oxide chains attached to a divinyl benzene core;

b) contacting a sample containing a ligate of interest under conditions sufficient to bind the ligate to the ligand;

c) removing any unbound proteins from the hydrogel-coated surface;

d) adjusting ionic strength of the sample to thereby remove the bound ligate from the hydrogel; and

e) collecting the separated ligates.

19. The method of Claim 18, wherein the support surface is selected from the group consisting of silica particles, porous polymeric material, polymeric film and ultrahigh molecular weight high density polyethylene.

20. The method of Claim 19, wherein the ligate is selected from the group consisting of macro-molecules, monoclonal antibodies, antigens, viruses and cells.

21. The method of Claim 20, wherein the cells are selected from the group consisting of blood platelets, white blood cells and endothelial cells.

22. The method of Claim 18, wherein the ligand is selected from the group consisting of antibodies, Protein A, F_{ab} fragments of antibodies, and active polysaccharides.

23. The method of Claim 22, wherein the active polysaccharide is heparin.

24. The method of Claim 22, wherein the ligand is monoclonal anti-Protein C IgG or F_{ab} fragments thereof.

25. A hydrogel comprising crosslinked polyethylene oxide star molecules having a plurality of hydroxy-terminated polyethylene oxide chains attached to a polymeric core.

26. The hydrogel of Claim 25, wherein the star molecules comprise from about 6 to about 50 hydroxy-terminated polyethylene oxide chains attached to a polymeric core that is divinyl benzene, wherein each chain has a molecular weight range of from 1000 to about 10,000.

27. A contact lens comprising crosslinked polyethylene oxide star molecules having a plurality of hydroxy-terminated polethylene oxide chains attached to a polymeric core, coated onto a suitable contact lens material.

28. A contact lens of Claim 27, wherein the star molecules comprise from about 6 to about 50 hydroxy-terminated polyethylene oxide chains attached to a polymeric core that is divinyl benzene, wherein each chain has a molecular weight range of from 1000 to about 10,000.

29. A contact lens made by the method of Claim 7.

30. A contact lens made by the method of Claim 11.

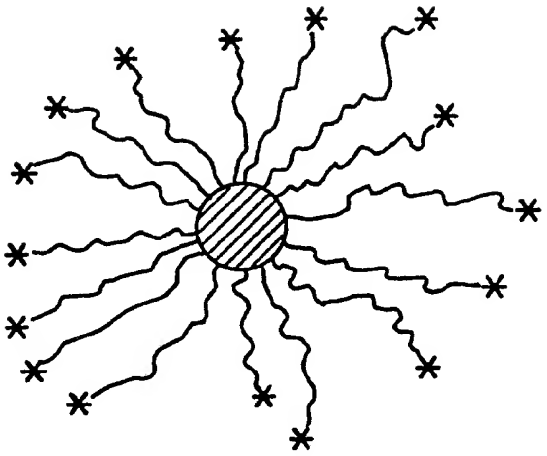

FIG. IA

LEGEND FOR FIG. IA & IB

= CROSS-LINKED DIVINYL BENZENE CORE

= PEO CHAIN

= HYDROXYL GROUP

= PST CHAIN

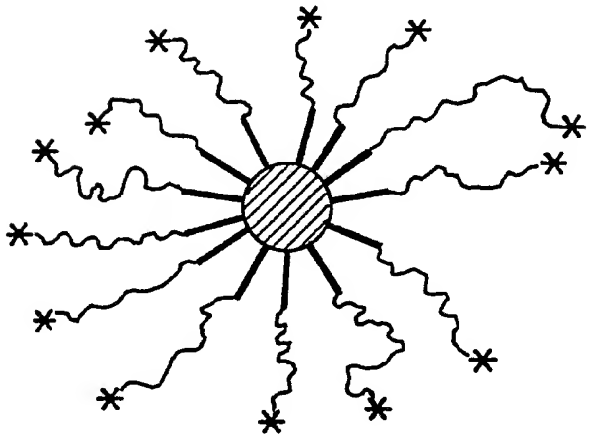

FIG. IB

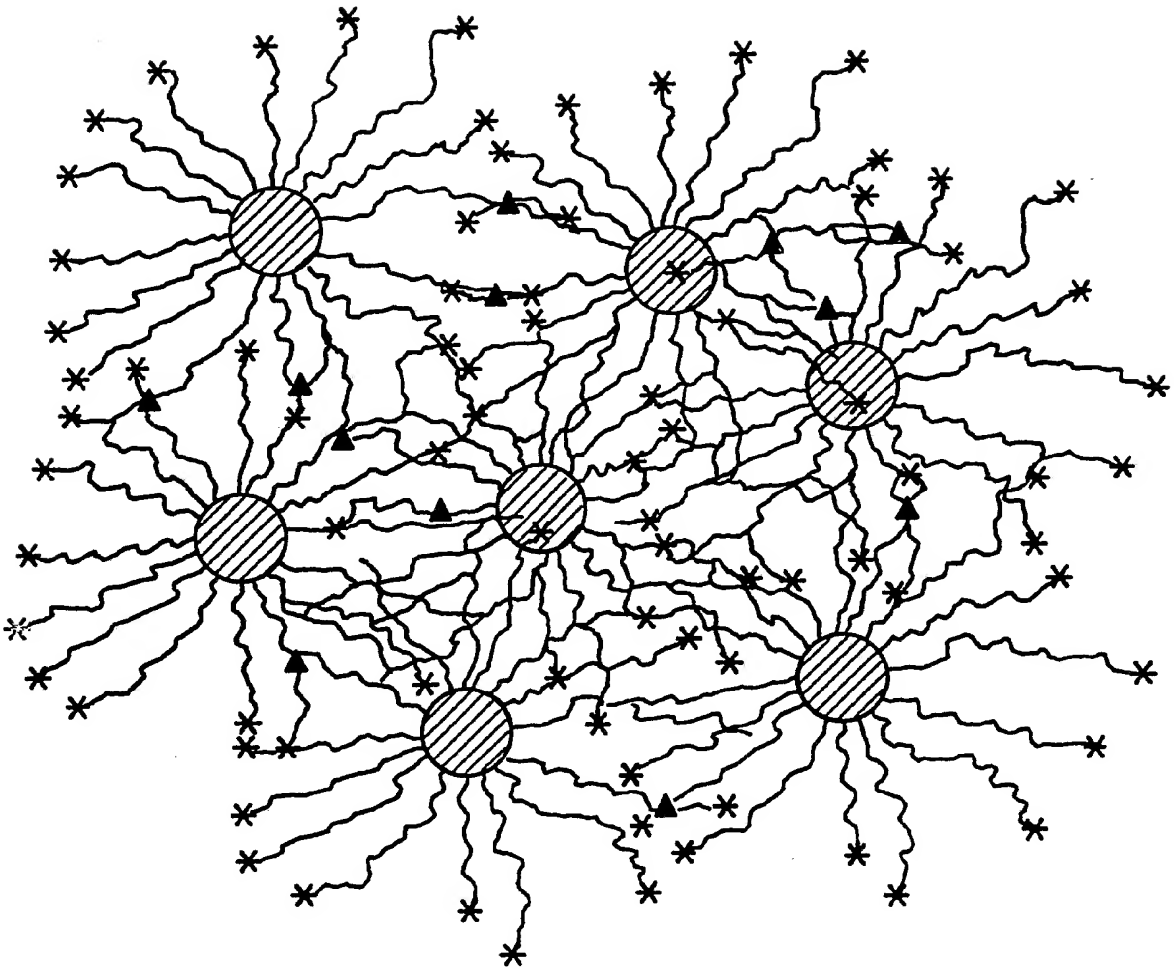

FIG. 2

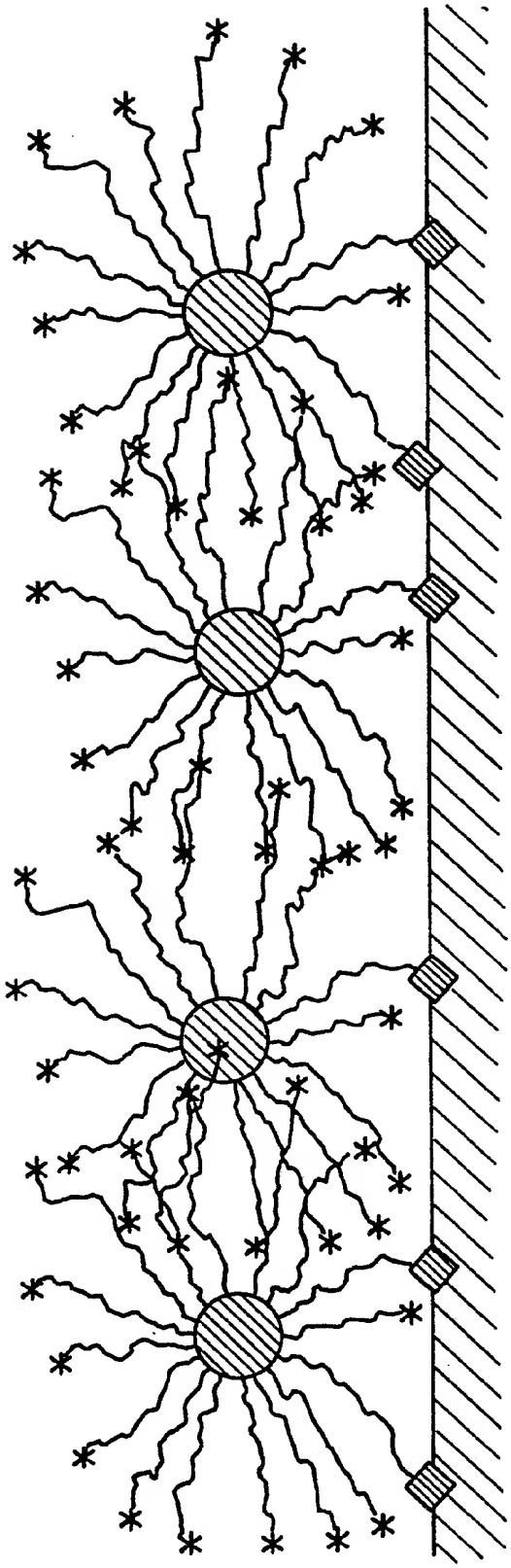
* = TRESYLATED HYDROXYL
= ATTACHMENT TO AMINO GROUP ON SUPPORT SURFACE

FIG. 3

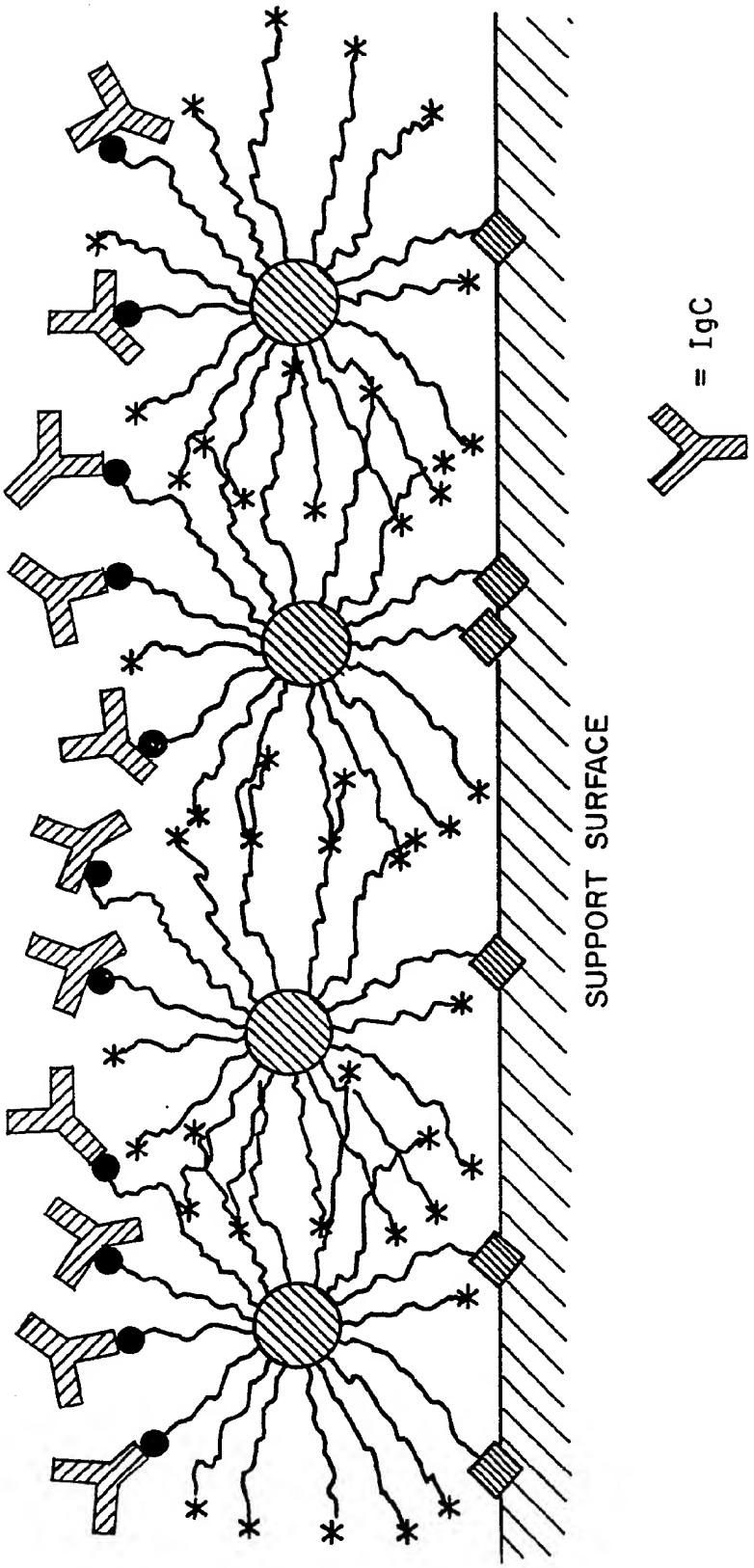

FIG. 4

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 91/01302

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC[5]: B 01 J 20/32, B 01 D 67/00, A 61 L 33/00, C 08 J 7/04

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC[5]	B 01 J, A 61 L, C 08 J

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
A	EP, A, 0156657 (CNRS) 2 October 1985 see page 4, line 3 - page 10, line 31	1,2,18
X	--	25,26
A	WO, A, 8602087 (YTKEMISKA INST.) 10 April 1986 see page 12, line 20 - page 15, line 25; pages 23-26	1,3,4,7, 8,10
	--	
A	EP, A, 0068509 (TORAY IND.) 5 January 1983 see page 7, line 25 - page 9, line 8; pages 17-18	1,7-10,27
	--	
A	US, A, 4280923 (SMALL) 28 July 1981 see column 8, example 14	18,19
	--	
	./.	

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
21st May 1991	17. 07. 91
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	Natalie Weinberg

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages	Relevant to Claim No.
A	EP, A, 0263184 (TORAY IND.) 13 April 1988 see page 4, line 1 - page 10, line 20; pages 22-23 --	1,2,15,16, 17
A	EP, A, 0332261 (STICHTING VOOR DE TECHN. WETENSCHAPPEN) 13 September 1989 --	
P,A	Chemical Abstracts, vol. 113, 8 October 1990, (Columbus, Ohio, US), P. Rempp et al.: "Anionically polymerized star macromolecules having divinylbenzene cores with grafted poly (ethylene oxide) arms as biomaterials", see abstract 133330f, & Polym. Prepr. (Am. Chem. Soc., Div. Polym. Chem.) 1990, 31(1), 215	1-6
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ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. US 9101302 SA 45422

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 03/07/91
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

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		AU-A- 3105589	14-09-89
		JP-A- 2017073	22-01-90
		US-A- 4965112	23-10-90